THE DEVELOPMENT OF THE HETEROTYPIC CHROMOSOMES IN POLLEN MOTHER CELLS.

D. M. MOTTIER.

(PRELIMINARY COMMUNICATION)

THE theory of a reduction division in the spore mother cells of higher plants has gained considerable ground in the past two or three years; so that at present probably the majority of observers, who have devoted themselves almost constantly to the problems of the chromosomes, seem firmly convinced that one of the two mitoses in the formation of the tetrad is a "reducing" division. There is still, on the other hand, some diversity of opinion, and several years may elapse before cytologists will be strictly in accord upon this the most difficult of cell problems.

One of the most important facts brought out by recent investigations is the shifting of the point in the tetrad formation, at which the qualitative separation of the chromosomes is held to occur, from the second, or homotypic mitosis, to the first or heterotypic division. Since it has been shown by FLEMMING and MEVES for the animal cell, and by GUIGNARD, STRASBURGER, and the writer, for the higher plants, that the daughter chromosomes of the heterotypic mitosis are split lengthwise as they separate in the metaphase, a more critical study has been devoted to the prophase of this division, and much light has been thrown upon certain obscure steps that have not as yet been satisfactorily explained. This double nature of the retreating chromosomes was regarded as a second longitudinal fission, since it could be seen that the spirem was double in a very early prophase. This apparent second longitudinal division seemed to have proved beyond any shadow of a doubt that the second mitosis is not a reducing division, as has been so insistently maintained by many zoologists. Now that it has been shown that the homotypic mitosis in the spore mother cells of plants is not a reduction division, the question to be answered is whether in the heterotypic division the chromosomes are bivalent, or whether the segments of each pair separate along the line of longitudinal fission.

Among those who are convinced of the bivalent character of the heterotype chromosomes, *i. e.*, that this is a reducing division, two views are held as to the manner in which these chromosomes are formed. According to the view advanced by STRASBURGER ('04) and FARMER ('05), the chromatin spirem splits longitudinally, the two segments or daughter spirems fusing again shortly afterwards, and segments into pieces equal to two somatic chromosomes placed end to end. Each piece, or double chromosome, folds, either during or after the cross segmentation, to form the familiar paired rods, rings, loops, etc., so often figured by the several observers. Consequently the two segments of each chromosome are not daughter chromosomes, formed by a longitudinal splitting, but two somatic chromosomes, each of which is split lengthwise; but, as stated above, this longitudinal split is not usually recognizable until the meta- or anaphase. JULES BERGHS ('04, '05) and other students of GRÉGOIRE assert that the longitudinal fission, so readily observed in the spirem of the prophase of the heterotypic mitosis, is not a real longitudinal fission of the chromatin thread, but that, during the contracted phase of the nucleus, the so-called "synapsis," the double spirem is formed by the approximation of two spirems, one being maternal and the other paternal. The chromosomes are therefore bivalent, and as they separate in metakinesis each splits lengthwise. BERGHS has studied *Convallaria majalis*, *Lilium speciosum*, *Allium fistulosum*, *Narthecium ossifragum*, *Helleborus foetidus*, and *Drosera rotundifolia*, and while he has presented an apparently closely connected series in the formation of the chromosomes, certain very important steps seem to have been omitted and others incorrectly interpreted.

STRASBURGER ('04) has based his conclusions upon a study of *Galtonia candicans*, a species claimed to be unusually favorable because of the small number of the chromosomes in the pollen mother cells, namely six. In this plant he finds that the spirem splits longitudinally in the early prophase of the heterotypic mitosis, as described by the writer several years ago, but the longitudinal splitting does not lead to the separation of the segments; so that later, as the spirem shortens and becomes thicker, no trace of this fission can be seen. The spirem now segments transversely into the six chromosomes, and each of these segments again, in a similar manner,

into two pieces of equal length. Thus arise twelve chromosomes which come together in pairs to form the bivalent chromosomes.

FARMER and MOORE ('05) in their joint publication have presented the results of their observations upon animals and plants. Of the latter the familiar and oft studied *Lilium candidum* heads the list, and to this form alone reference will here be made. As has frequently been described, the chromatin ribbon of *Lilium candidum* splits longitudinally, and the halves usually separate more or less widely from each other. Later the halves reapproximate, and the split closes up again. At the same time the entire spirem shortens and thickens. The contraction goes on rapidly, and the original longitudinal split soon ceases to be noticeable, being visible in exceptionally favorable cases only. A rearrangement of the thread now sets in, such that parts of the spirem become pulled into parallel positions. This is well seen in those places where, at the bend of a convolution, an attachment to the nuclear membrane has taken place. In this manner, a close and parallel approximation of lengths of the entire spirem is effected; and this parallel arrangement, it is stated, has been commonly interpreted as representing the parallel split halves of the spirem thread. As a consequence of this rearrangement of the spirem, or parts which give rise to chromosomes, the segments when isolated very often exhibit the form of a loop, open at one end, with sides either parallel to each other, or more frequently twisted over one another. All chromosomes are not formed in this way however. Sometimes two, more or less straight, rodlets may unite so as to give rise to figures of rings, ellipses, etc. The point especially emphasized by the joint authors is "that the two rods, sides of loops, or whatever other form the structure as a whole may assume, represent, not the longitudinal halves of a split thread, but the approximation of serially distinct regions of the spirem as a whole. Thus each heterotypic chromosome is a bivalent structure, and their reduced number is due to the approximation and more or less intimate, though temporary, union of the equivalents of pairs of somatic chromosomes."

ALLEN ('05), who has made a very detailed study of this mitosis in *Lilium canadense*, does not conclude in favor of a reducing division. He interprets his results as indicating that a longitudinal

division of the chromatin precedes each of the two mitoses, the second fission occurring during the meta- or anaphase of the first, or heterotypic division.

In my first study of Podophyllum, in 1896, certain steps in the development of the heterotypic chromosomes were never clearly understood, and at that time the phenomena in question were attributed to poor fixation. On the appearance of STRASBURGER'S paper on *Galtonia candicans*, I again took up the study of Podophyllum, because of the facts just stated, and because the reduced number of chromosomes in this plant is only eight. This study has resulted in a clearer understanding of the phenomena in question, and for comparison my study is being extended to other plants, among them being *Lilium candidum*. Although my study is not yet completed, and owing to the delay in publication which may ensue, it has been thought best to make public a brief statement of the conclusions reached in reference to the heterotypic chromosomes in the pollen mother cells of *Podophyllum peltatum*.

It may be stated at the outset that my earlier description of the resting nucleus in the pollen mother cell of this plant is substantially correct. The fine linin network contains many small chromatin granules of uniform size and distribution. One or more nucleoli may be present. Following this comes the contracted condition, or synapsis. In my earlier publication this condition was regarded as being due to the action of reagents, but I am now convinced that the phenomenon is normal. Following synapsis, the loosening up of the contracted ball results in the chromatin spirem; and as soon as the spirem has emerged from the balled-up condition, or shortly afterwards, and has assumed a more regular arrangement, it is clearly seen to be split longitudinally. I am not, at this writing, prepared to state definitely whether the spirem is formed double as claimed by BERGHS and ALLEN, but in the contracted condition portions of the thread which occasionally extend out free from the mass as a loop could sometimes be seen to be double, or appearing as if split lengthwise. After all this is not strange, when we remember that the spirem is derived from a network, and nothing is more probable than that in the transformation of the net into the thread parallel threads of consecutive meshes would be approximated. It seems to

me now, though the statement is made with reserve, that the double nature of the spirem at this stage, referred to by BERGHS and ALLEN is due to the phenomenon just stated, and not to the approximation of two distinct spirems.

Omitting details, the next step of importance is the clear and unmistakable manifestation of the longitudinal splitting of the chromatin spirem. In Podophyllum the segments of the spirem do not divaricate as in *Lilium candidum*, for example, but frequently parts of the two daughter spirems do separate for considerable stretches. The segments are more or less twisted about each other. Following this stage, the spirem shortens and thickens somewhat, and the longitudinal fission becomes less and less distinct, and finally almost every trace of the double nature of the thread disappears. The thread does not shorten nor thicken as rapidly, nor to the extent that it is usually supposed to shorten and thicken, before its transverse segmentation into chromosomes, and it is just at this point that the writer and many others have been led into error. The thread does, of course, shorten and thicken to some extent, and as a result its arrangement reaches its greatest regularity. This is the stage of the loose or hollow spirem so frequently observed. However, there is no well-marked regularity in the convolutions of the spirem throughout its entire length; some of its turns follow the nuclear periphery, while others traverse the interior. In the nuclear cavity the turns are often short and kinked. In sections including the whole nucleus, it is not possible to follow accurately the entire thread, but it seems that there are few or no free ends, and very rarely is any trace of a longitudinal split discernible.

The stage of the loose and more regular spirem seems to persist for some time, as it is frequently met with in the preparations. The next step in the prophase has been one of the stumbling-blocks of cytologists, and it is the one that the writer ascribed to poor fixation in his earlier studies. It may be true that this stage is difficult of fixation, and that, together with its short duration, has probably been the main reason for the failure to understand its true significance. Just before the transverse segmentation of the spirem and the final differentiation into the chromosomes, the loose spirem loses the regular arrangement it may have had and undergoes a contraction

such that there is a parallel approximation of certain parts of the spirem to form long loops; while other parts, especially those near the center of the nuclear cavity, become knotted and entangled. In the closely contracted and entangled parts of the spirem it is not possible to make out clearly and definitely the arrangement of the chromatin thread, but there is no doubt as to the true nature of the longer loops. Sometimes the loops show a tendency to radiate from the more contracted entanglement of the spirem. The arrangement of these loops is very rarely so regular as figured by FARMER for *Lilium candidum* (*l. c., fig. 9*). The parallel sides of the loops are usually twisted upon each other, and the bend of the loop is often, though not always, toward the periphery of the nucleus. It is during this contracted and entangled condition that the thread segments, either partly or completely into the chromosomes. After segmentation the chromosomes begin to contract and thicken more rapidly, and as a result they become more scattered in the nuclear cavity, so that the relation of the two segments toward each other can be readily made out. It is in this and the spindle stage that the chromosomes have been most frequently figured. Those which show the greatest regularity give the impression that they have been formed by a long piece of the spirem folding over in the form of a loop and the parallel sides of the loop twisting upon each other. Others appear as two parallel rods, which may or may not be twisted upon each other; and in still others the two segments are variously oriented toward each other, as has been figured time and again, and in the greatest profusion, by the different observers.

When one considers the chromosomes in this stage and the longitudinally split spirem of the early prophase, the most natural conclusion is this, namely, that the two parallel rods, or the two segments of each chromosome, of whatever shape, represent adjacent and parallel parts of the longitudinally split spirem; that the spirem thus split merely contracted and shortened, so that the two rather thick halves of each chromosome seemed to owe their thickness to contraction and shortening alone. As a matter of fact, however, the longitudinal split of the thread in Podophyllum becomes obliterated during the formation of the loose and more regular spirem, so that scarcely a trace of the fission can be seen; and, as previously stated

in the foregoing, the spirem contracts and thickens much less before its cross segmentation than has been supposed. The greatest contraction occurs after segmentation, and furthermore the two segments, or rods, of each chromosome do not represent the parallel halves of the longitudinally split spirem, but the approximation of serially distinct parts of the spirem as a whole. Each half of the chromosome is consequently double, resulting from the early longitudinal fission of the spirem, and this fission manifests itself during the meta- and anaphase. It is, therefore, the original longitudinal fission which has been regarded as a second longitudinal splitting. The heterotypic chromosomes of Podophyllum, therefore, are bivalent, and the first mitosis in the pollen mother cells is a "reducing" division. This seems to me now to be the only proper interpretation of the heterotypic chromosomes in Podophyllum. The writer has been reluctant to give up the theory that a longitudinal fission occurs for each mitosis, and he has done so only after a long and careful study of many preparations.

INDIANA UNIVERSITY, BLOOMINGTON.

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